

Description of a new species of the Asian newt genus *Tylototriton* Anderson, 1871 (Urodela, Salamandridae) from central China

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Abstract

Based on morphological comparisons and molecular phylogenetic analyses, a new species of the genus *Tylototriton* from Wufeng County, Hubei Province, China, is described. Phylogenetic analyses of mitochondrial 16S rRNA and ND2 gene sequences supported the new species as an independent clade, sister to a clade containing *Tylototriton dabienicus* and *T. gaowangjienensis*. The uncorrected p-distances of the 16S rRNA and ND2 genes between the new species and its closest congeners were 0.7% and 1.7%, respectively. Divergence time estimates indicated separation from *T. dabienicus* and *T. gaowangjienensis* at 2.53 million years ago (Mya; 95% highest posterior density [HPD]: 1.53–3.72 Mya). Bayesian species delimitation (BFD and bPTP analyses) strongly supported the new taxon as a distinct species. Morphologically, it is distinguished by: (1) moderate body size (SVL 63.7–77.2 mm in males, 74.9–88.1 mm in females); (2) head width exceeding head length; (3) presence of a gular fold; (4) tail shorter than snout–vent length; (5) orange distal digits, cloacal periphery, and tail margin; (6) limb tips meeting when fore- and hindlimbs are pressed along the trunk; (7) fingertips extending beyond the snout when forelimbs are stretched forward; and (8) continuous, indistinct, nodule-like warts on the body sides.

Key Words

morphology, molecular phylogenetic analysis, new species, *Tylototriton*

Introduction

The newt genus *Tylototriton* Anderson, 1871, is widely distributed from the Sikkim and Darjeeling areas of north-central India and Nepal eastward and southeastward to central China, non-peninsular regions of Myanmar and Thailand, and through Laos to the central and northern highlands of Vietnam, southern China, and Hainan Island (Frost 2025). Taxonomic assignments of

the group have been highly controversial for decades (Fei et al. 2005, 2012; Dubois and Raffaëlli 2009; Fei and Ye 2016; Poyarkov et al. 2021, 2024; Lyu et al. 2021). Early classifications by Fei et al. (2005) divided the genus into two species groups, the *T. verrucosus* and *T. asperrimus* groups. Dubois and Raffaëlli (2009) elevated these groups to subgenera (*Tylototriton* and *Yaotriton*), while Fei et al. (2012) reclassified *Tylototriton* sensu lato into three genera, *Tylototriton* sensu stricto, *Yaotriton*,

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and *Liangshantriton*, based on morphological and ecological differences. Fei and Ye (2016) further subdivided *Tylototriton* sensu stricto into two subgenera (*Tylototriton* and *Qianotriton*). However, molecular phylogenetic analyses revealed unresolved relationships among major clades, prompting Wang et al. (2018) to advocate retaining the genus as *Tylototriton* sensu lato. Subsequent studies, such as Lyu et al. (2021), proposed dividing the genus into three species groups (*T. asperrimus*, *T. taliangensis*, and *T. verrucosus*), whereas Poyarkov et al. (2021, 2024) retained the subgenera *Yaotriton* and *Tylototriton* based on mitochondrial phylogenies.

Historically, species diversity within *Tylototriton* was underestimated due to the genus' conserved morphology. Many species share similar body plans, color patterns, and skeletal structures, leading to misidentifications and taxonomic ambiguities. For example, populations in Guizhou Province, China, were initially identified as *T. asperrimus* or *T. wenxianensis* based on morphology alone (Wu et al. 1986), but molecular data later revealed cryptic lineages requiring formal description (Li et al. 2020; Luo et al. 2022).

The integration of molecular data has revolutionized *Tylototriton* taxonomy. Wang et al. (2018) uncovered extensive cryptic diversity using mitochondrial and nuclear genes, with 18 new species described in the past 5 years (Frost 2025). For instance, *T. dabienicus* lineage 2 was named *T. anhuiensis* (Qian et al. 2017), *T. wenxianensis* lineage 2 became *T. maolanensis* (Li et al. 2020), and *T. asperrimus* lineage 2 was designated *T. sini* (Lyu et al. 2021). These revisions clarify evolutionary relationships and inform conservation strategies.

During an amphibian survey in Wufeng County, Hubei Province, China, in May 2024, we collected eight newt specimens. Phylogenetic analyses revealed their close affinity to *Tylototriton wenxianensis* lineage 3, a cryptic clade identified by Wang et al. (2018). By integrating morphological comparisons and genetic divergence metrics, we herein describe these specimens as a new species, further highlighting the hidden diversity within this taxonomically complex genus.

Material and methods

Sampling

Eight specimens, containing five males and three females of the unnamed taxon, were collected from Hubei Province, China (Fig. 1). All specimens were fixed in 10% buffered formalin for 24 h and then transferred to 75% ethanol. Tissue samples were preserved separately in 95% ethanol prior to fixation. Specimens collected in this work were deposited in the Chengdu Institute of Biology (CIB), Chinese Academy of Sciences (CAS). The Animal Care and Use Committee of CIB, CAS, provided full approval for this research (Number: CIB2023041103).

Collection of molecular data

DNA was extracted from muscle tissue using a DNA extraction kit from Tiangen Biotech Co., Ltd. (Beijing). A fragment encoding partial 16S rRNA (16S) and NADH dehydrogenase subunit 2 (ND2) genes was amplified and sequenced. The primers used for 16S were P7 (5'-CG-CCTGTTTACCAAAAACAT-3') and P8 (5'-CCGGTCT-GAACTCAGATCACGT') following Simon et al. (1994), and for ND2 were SL-1 (5'-ATAGAGGTTCAAAC-CCTCTC-3'), and SL-2 (5'-TTAAAGTGTCTGGGTTG-CATTCAG-3') following Wang et al. (2018). PCR amplification reactions were performed under the following conditions: an initial denaturing step at 95 °C for 4 min; 35 cycles of denaturing at 95 °C for 40 s, annealing at 50 °C (for 16S) or 52 °C (for ND2) for 40 s, and extension at 72 °C for 70 s; and a final extension step at 72 °C for 10 min. PCR products were purified with spin columns and then sequenced with both forward and reverse primers, the same as those used for PCR. Sequencing was conducted using an ABI Prism 3730 automated DNA sequencer at Chengdu TSING KE Biological Technology Co., Ltd. (Chengdu, China). All sequences were deposited in GenBank (for GenBank accession numbers, refer to Table 1).

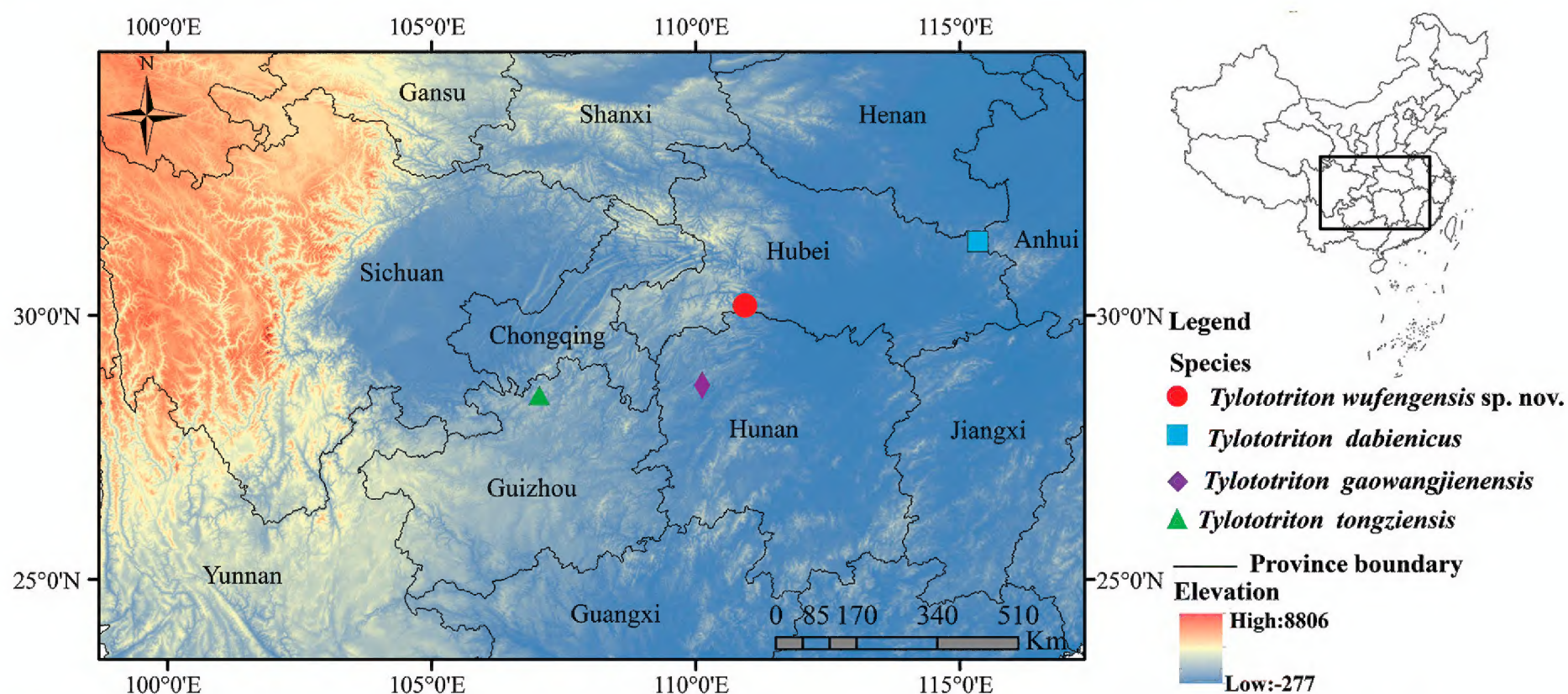


Figure 1. Map showing the distribution sites of *Tylototriton wufengensis* sp. nov., *T. dabienicus*, *T. gaowangjienensis*, and *T. tongziensis*.

Table 1. Information for samples used in molecular phylogenetic analyses in this study; a slash (/) indicates information absent.

ID	Species	Voucher	Locality	GenBank accession number	
				16S	ND2
1	<i>T. wufengensis</i> sp. nov.	CIB WH10007	Wufeng Co., Hubei Prov., China	KY800603	KY800866
2	<i>T. wufengensis</i> sp. nov.	CIB WH10003	Wufeng Co., Hubei Prov., China	KY800602	KY800865
3	<i>T. wufengensis</i> sp. nov.	CIB WH10002	Wufeng Co., Hubei Prov., China	KY800601	KY800864
4	<i>T. wufengensis</i> sp. nov.	CIB WF20240502008	Wufeng Co., Hubei Prov., China	PX625474	PX549138
5	<i>T. wufengensis</i> sp. nov.	CIB WF20240502007	Wufeng Co., Hubei Prov., China	PX625478	PX549142
6	<i>T. wufengensis</i> sp. nov.	CIB WF20240502006	Wufeng Co., Hubei Prov., China	PX625479	PX549143
7	<i>T. wufengensis</i> sp. nov.	CIB WF20240502005	Wufeng Co., Hubei Prov., China	PX625473	PX549137
8	<i>T. wufengensis</i> sp. nov.	CIB WF20240502003	Wufeng Co., Hubei Prov., China	PX625477	PX549141
9	<i>T. wufengensis</i> sp. nov.	CIB WF20240502002	Wufeng Co., Hubei Prov., China	PX625476	PX549140
10	<i>T. wufengensis</i> sp. nov.	CIB WF20240502004	Wufeng Co., Hubei Prov., China	PX625472	PX549136
11	<i>T. wufengensis</i> sp. nov.	CIB WH10001	Wufeng Co., Hubei Prov., China	KY800600	KY800863
12	<i>T. wufengensis</i> sp. nov.	WF20240502001	Wufeng Co., Hubei Prov., China	PX625475	PX549139
13	<i>T. dabienicus</i>	HNNU 1004-024	Shangcheng Co., Henan Prov., China	KY800608	KC147812
14	<i>T. dabienicus</i>	HNNU 1004-015	Shangcheng Co., Henan Prov., China	KY800607	KC147811
15	<i>T. dabienicus</i>	HNNU 1004-026	Shangcheng Co., Henan Prov., China	KY800609	KY800869
16	<i>T. gaowangjienensis</i>	HNNU 20210425001	Guzhang Co., Henan Prov., China	PP090893	PP092977
17	<i>T. gaowangjienensis</i>	HNNU 20210508001	Guzhang Co., Henan Prov., China	PP090894	PP092978
18	<i>T. gaowangjienensis</i>	HNNU 20210504001	Guzhang Co., Henan Prov., China	PP090895	PP092979
19	<i>T. tongziensis</i>	CIB TZ20160714002	Tongzi Co., Guizhou Prov., China	OK338498	OK349413
20	<i>T. tongziensis</i>	CIB TZ20160714010	Tongzi Co., Guizhou Prov., China	OK338499	OK349414
21	<i>T. tongziensis</i>	CIB WB2020202	Tongzi Co., Guizhou Prov., China	OK338500	OK349415
22	<i>T. tongziensis</i>	CIB WB2020190	Tongzi Co., Guizhou Prov., China	OK338495	OK349410
23	<i>T. tongziensis</i>	CIB WB2020081511	Tongzi Co., Guizhou Prov., China	OK338496	OK349411
24	<i>T. tongziensis</i>	CIB WB2020191	Tongzi Co., Guizhou Prov., China	OK338497	OK349412
25	<i>T. anhuiensis</i>	CIB 08042905-3	Yuexi Co. Anhui Prov., China	KY800588	KY800854
26	<i>T. anhuiensis</i>	CIB 08042905-4	Yuexi Co. Anhui Prov., China	KY800589	KY800855
27	<i>T. maolanensis</i>	CIB ML20180427003	Libo Co., Guizhou Prov., China	MK835789	MK820701
28	<i>T. maolanensis</i>	CIB ML20180427004	Libo Co., Guizhou Prov., China	MK835790	MK820702
29	<i>T. broadoridgus</i>	CIB 200084	Sangzhi Co., Hunan Prov., China	KY800570	KY800837
30	<i>T. daloushanensis</i>	CIB WG200600019	Suiyang Co., Guizhou Prov., China	KY800544	KY800817
31	<i>T. daloushanensis</i>	CIB WG20060007	Suiyang Co., Guizhou Prov., China	KY800545	KY800818
32	<i>T. daloushanensis</i>	CIB WG20090601002	Suiyang Co., Guizhou Prov., China	KY800573	KY800840
33	<i>T. daloushanensis</i>	CIB WG20090601001	Suiyang Co., Guizhou Prov., China	KY800574	KY800841
34	<i>T. wenxianensis</i>	CIB 20090527	Wenxian Co., Gansu Prov., China	KY800579	KC147813
35	<i>T. wenxianensis</i>	CIB 20070639	Qingchuan Co., Sichuan, China	KY800542	KY800815
36	<i>T. wenxianensis</i>	CIB 2010123101	Pingwu Co., Gansu, China	KY800604	KY800867
37	<i>T. wenxianensis</i>	CIB 2010123102	Pingwu Co., Gansu, China	KY800605	KY800868
38	<i>T. liuyangensis</i>	CIB 110601F06	Liuyang City, Hunan Prov., China	KY800615	KY800875
39	<i>T. lizhanchang</i>	KUHE:42316	Yizhang Co., Hunan Prov., China	KY800621	KY800881
40	<i>T. vietnamensis</i>	IEBRA.0701	Mauson, Lang Son Prov., Vietnam	KY800613	KY800873
41	<i>T. panhai</i>	NUOL 00421	/	/	KT304310
42	<i>T. pasmansi</i>	IEBR 4466	Da Bac, Hoa Binh, Vietnam	/	MT210166
43	<i>T. sparreboomi</i>	IEBR 4476	Sin Ho, Lai Chau, Vietnam	/	MT210167
44	<i>T. asperrimus</i>	CIB GX20080714	Jinxiu Co., Guangxi Prov., China	KY800546	KY800819
45	<i>T. thaiorum</i>	ZMMU A-7577	Pu Hoat NR, Nghe An, Vietnam	MW883482	MW883478
46	<i>T. notialis</i>	FMNH:HERP:271120	Boualapha, Khammouan, Laos	/	HM462061
47	<i>T. sini</i>	SYS a008354	Mt Yunkai, Guangdong Prov., China	/	OK539836
48	<i>T. sini</i>	SYS a008353	Mt Yunkai, Guangdong Prov., China		OK539835
49	<i>T. hainanensis</i>	CIB 20081048	Mt. Diaoluo, Hainan Prov., China	KY800553	KC147817
50	<i>T. ziegleri</i>	VNMIN 3390	Quan Ba, Ha Giang, Vietnam	KY800625	KY800889
51	<i>T. koliaensis</i>	ROM26520	Quang Thanh, Nguyen Binh, Cao Bang, Vietnam	/	JQ046351
52	<i>T. zaimeng</i>	MZMU2947	Zaimeng Lake, Kangpokpi Dist., Manipur, India	/	OR039162

ID	Species	Voucher	Locality	GenBank accession number	
				16S	ND2
53	<i>T. zaimeng</i>	MZMU2948	Zaimeng Lake, Kangpokpi Dist., Manipur, India	/	OR039163
54	<i>T. zaimeng</i>	MZMU2936	Phungyar, Kamjong Dist., Manipur, India	/	OR039165
55	<i>T. panwaensis</i>	CAS 245418	Kachin state, Myanmar	/	KT304279
56	<i>T. podichthys</i>	NCSM 77725	/	/	KT304295
57	<i>T. houi</i>	Thoui02	Yulong, Lijiang, Yunnan Prov., China	ON008212	/
58	<i>T. houi</i>	Thoui01/MZL46960	Yulong, Lijiang, Yunnan Prov., China	ON008211	/
59	<i>T. shanjing</i>	CIB 980004	Baoshan City, Yunnan Prov., China	KY800562	KY800831
60	<i>T. verrucosus</i>	CIB-TSHS1	Longchuan Co., Dehong state, Yunnan Prov., China	KY800581	KY800847
61	<i>T. pulcherrimus</i>	KUHE:46406	Pet Trade	KY800620	KY800880
62	<i>T. uyenoi</i>	KUHE:19147	Doi Suthep, Chiang Mai, Thailand	/	AB830733
63	<i>T. soimalai</i>	CUMZ-A-8253	Mae Tuen Wildlife Sanctuary, Tak, Thailand	/	PQ218721
64	<i>T. umphangensis</i>	CUMZ-A-8243		/	OK092618
65	<i>T. anguliceps</i>	NUOL 00420	/	/	KT304301
66	<i>T. phukhaensis</i>	CUMZ-A-7717	DPKNP, Nan, Thailand	/	MN912573
67	<i>T. yangi</i>	KUHE:42282	Pet Trade	KY800624	KY800887
68	<i>T. shanorum</i>	KUHE 42348	Shan State, Myanmar	/	AB769544
69	<i>T. ngarsuensis</i>	LSUHC13762	Shan State, Myanmar	/	MH836585
70	<i>T. himalayanus</i>	CIB 201406246	Mai Pokhari, Illam, Mechi,Nepal	KY800590	KT765173
71	<i>T. kachinorum</i>	ZMMU A5953	Ingyin Taung Mt., Indawgyi, Kachin, Myanmar	MK095618	/
72	<i>T. kweichowensis</i>	CIB Wg20080818014	Bijie City, Guizhou Prov., China	KY800551	KY800823
73	<i>T. pseudoverrucosus</i>	CIB WCG2012003	Ningnan Co., Liangshanyizu state, Sichuan Prov., China	KY800597	KY800861
74	<i>T. taliangensis</i>	CIB GG200110183	Shimian Co., Yan'an City, Sichuan Prov., China	KY800559	KC147819
75	<i>Echinotriton chinhaiensis</i>	CIB ZHJY2	Zhenhai Co., Zhejiang Prov., China	KY800628	KY800892
76	<i>Pleurodeles waltl</i>	/	Cadiz, Andalusia, Spain	EU880330	EU880330
77	<i>Pachytriton labiatus</i>	/	/	EU880325	EU880325

For phylogenetic analyses, we downloaded corresponding sequences for all related species from GenBank according to previous studies (for GenBank accession numbers, refer to Table 1).

Phylogenetic analyses and divergence time estimation

For phylogenetic analyses, we newly generated eight sequences each for the 16S and ND2 genes of the undescribed species. Additionally, we downloaded 117 corresponding sequences from GenBank, including 50 sequences of 16S (covering 26 known *Tylototriton* species and three outgroups) and 67 sequences of ND2 (covering 39 known *Tylototriton* species and three outgroups). In total, our dataset included 41 of the 43 currently recognized species of *Tylototriton*, as well as three outgroup taxa (*Pleurodeles waltl*, *Pachytriton labiatus*, and *Echinotriton chinhaiensis*).

Sequences were assembled and aligned using the ClustalW module in BioEdit 7.0.9.0 (Hall 1999) with default settings. Based on the 16S + ND2 concatenated dataset, phylogenetic analyses were conducted using ML and BI methods, implemented in PhyML 3.0 (Guindon et al. 2010) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), respectively. The best-fit model was obtained using the Bayesian information criterion (BIC) computed with PartitionFinder 2 (Lanfear et al. 2012). The analysis suggested

that the best partition scheme comprised the 16S and ND2 genes. We selected GTR + R + T as the best model for 16S and TN93 + I + G as the best model for the ND2 gene. For ML analyses, the bootstrap consensus tree inferred from 1,000 replicates was used to estimate nodal support for inferred relationships on the phylogenetic trees. For BI analyses, four Markov chains were run for 50 million generations, with sampling every 1,000 generations, until the trees reached convergence (average standard deviation of split frequencies < 0.05). The first 25% of trees were removed as burn-in, followed by calculation of Bayesian posterior probabilities and construction of the 50% majority-rule consensus tree from post-burn-in samples. Finally, uncorrected p-distances (1,000 replicates) between species, based on 16S (27 species) and ND2 (40 species), were calculated in MEGA 6.06 (Tamura et al. 2013).

Based on the 16S + ND2 concatenated dataset, we estimated lineage divergence times using a relaxed molecular clock model with a Yule tree prior in BEAST 1.8.4 (Drummond et al. 2012). In this analysis, *Pleurodeles* Michahelles, 1830 and *Tylototriton* sensu lato were calibrated at 44 million years ago (Ma) as the primary calibration constraint, whereas the divergence time of the most recent common ancestor (TMRCA) of the genus *Tylototriton* was inferred to be 14.0 Ma (95% HPD: 11.8–16.6) as the secondary constraint (Wang et al. 2018). Calibration nodes used a lognormal prior distribution. BEAST was run three times for 1 × 10⁹ generations for robustness, producing similar results

across runs and yielding effective sample size (ESS) values greater than 200 for all parameters, as calculated in Tracer v1.7 (Rambaut et al. 2018). A maximum clade credibility (MCC) tree was generated using TreeAnnotator v2.4.1 (Drummond and Rambaut 2007), with a 10% burn-in and a posterior probability threshold of 0.5.

Species delimitation

To assess whether the undescribed species represents a distinct species, we applied two complementary approaches: a Bayesian hypothesis-testing framework (Bayes factor delimitation, BFD; Gummer et al. 2014) and the Poisson tree processes model (bPTP; Zhang et al. 2013), using the 16S + ND2 concatenated dataset.

For BFD, we tested two species delimitation models in BEAST 1.8.4 (Drummond et al. 2012), one treating the undescribed species as a separate species (nine-species model) and another merging it with *T. dabienicus* and *T. gaowangjienensis* (seven-species model). Analyses used an uncorrelated lognormal relaxed clock, a Yule process for the species tree prior, and a piecewise linear plus constant root population model. Each model was run twice for 1×10^9 MCMC generations (sampling every 1,000 steps; 25% burn-in). Marginal likelihoods were estimated using path-sampling (PS) analyses (100 steps, 1×10^6 generations each). Support for competing models was evaluated following Bayes factor criteria.

For bPTP analyses, we analyzed the BI gene tree using the bPTP web server (<http://species.h-its.org/ptp/>), running 1×10^5 generations (thinning = 100, burn-in = 10%). Convergence was confirmed by inspection of log-likelihood traces.

Morphological comparisons

Morphological measurements were made with dial calipers to the nearest 0.1 mm by Shengchao Shi, following Li et al. (2022). A total of 18 adult specimens were examined and measured for 20 morphometric characters, comprising eight specimens of the undescribed species (five males and three females), four specimens of *T. dabienicus* (two males and two females), and six specimens of *T. tongziensis* (four males and two females) from its type locality. Additionally, morphological data for *T. gaowangjienensis* were obtained from Huang et al. (2024), which included measurements from five males and seven females (Suppl. material 1), abbreviated as follows:

ED	eye diameter (distance from the anterior corner to the posterior corner of the eye);
FIIL	the third finger length (distance from base to tip of finger III);
HDL	head length (distance from the tip of the snout to the articulation of jaw);
HDW	maximum head width (greatest width between the left and right articulations of the jaw);

IAE	(distance between posterior corners of eyes);
IFE	(distance between anterior corners of eyes);
IND	internasal distance (minimum distance between the inner margins of the external nares);
IOD	interorbital distance (minimum distance between the inner edges of the upper eyelids);
LLA	length of lower arm (distance from elbow to tip of wrist);
ML	manus length (distance from tip of third digit to proximal edge of inner palmar tubercle);
SL	snout length (distance from the tip of the snout to the anterior corner of the eye);
SVL	snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
TAL	tail length (from posterior margin of the anal opening to the tip of the tail);
TH	tail height (maximum tail height);
THIL	the third toe length (distance from base to tip of toe III);
TL	tibia length (distance from knee to tarsi);
TOL	total length (distance from tip of snout to tip of tail);
TRL	trunk length (from gular fold of throat to anterior tip of vent);
UEW	upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

To reduce the impact of allometry, size-corrected values were calculated as the ratio of each character to SVL for subsequent morphometric analyses. Principal component analysis (PCA) of size-corrected variables and simple bivariate scatterplots were used to explore and illustrate morphometric differences between the undescribed taxon and closely related species, including *T. dabienicus* and *T. tongziensis*. One-way analysis of variance (ANOVA) was used to test the significance of differences in morphometric characters between the undescribed taxon and *T. dabienicus* and *T. tongziensis* in males. Statistical analyses were performed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA), and differences were considered significant at $p < 0.05$.

We compared the morphological characters of the undescribed taxon with those of other species of *Tylototriton*. Comparative data were obtained from the literature for 43 species of *Tylototriton* (Table 2).

Results

Phylogenetic analyses

The aligned sequence matrix of the 16S and ND2 genes contained 511 and 1,032 bp, respectively. Both BI and ML analyses recovered the specimens of the undescribed taxon clustered into an independent clade, sharing a sister relationship with the clade containing *T. dabienicus* and *T. gaowangjienensis* (Fig. 2). The uncorrected ND2 p-distances between the unnamed taxon and its most

Table 2. References for morphological characters of congeners of the genus *Tylototriton*.

ID	Species	Literature obtained
1	<i>T. anguliceps</i> Le, Nguyen, Nishikawa, Nguyen, Pham, Matsui, Bernardes & Nguyen, 2015	Le et al. 2015
2	<i>T. anhuiensis</i> Qian, Sun, Li, Guo, Pan, Kang, Wang, Jiang, Wu & Zhang, 2017	Qian et al. 2017
3	<i>T. asperrimus</i> Unterstein, 1930	Fei et al. 2006; Fei and Ye 2016
4	<i>T. broadoridgus</i> Shen, Jiang & Mo, 2012	Shen et al. 2012; Fei and Ye 2016
5	<i>T. dabienicus</i> Chen, Wang & Tao, 2010	Chen et al. 2010; Fei and Ye 2016
6	<i>T. daloushanensis</i> Zhou, Xiao & Luo, 2022	Luo et al. 2022
7	<i>T. gaowangjienensis</i> Huang, Xiang, Zhang, Wu, & Jiang, 2024	Huang et al. 2024
8	<i>T. hainanensis</i> Fei, Ye & Yang, 1984	Fei et al. 1984; Fei and Ye 2016
9	<i>T. himalayanus</i> Khatiwada, Wang, Ghimire, Vasudevan, Paudel & Jiang, 2015	Khatiwada et al. 2015
10	<i>T. houi</i> Hernandez & Dufresnes, 2022 “2023”	Dufresnes and Hernandez 2022
11	<i>T. joe</i> Rao, Zeng, Zhu & Ma, 2022 “2020”	Rao 2022
12	<i>T. kachinorum</i> Zaw, Lay, Pawangkhanant, Gorin & Poyarkov, 2019	Zaw et al. 2019
13	<i>T. koliaensis</i> Poyarkov, Nguyen, Le, Le, Arkhipov, Gorin, Hernandez, & Dufresnes, 2024	
14	<i>T. kweichowensis</i> Fang & Chang, 1932	Fei et al. 2006; Fei and Ye 2016
15	<i>T. liuyangensis</i> Yang, Jiang, Shen & Fei, 2014	Yang et al. 2014; Fei and Ye 2016
16	<i>T. lizhenchangi</i> Hou, Zhang, Jiang, Li & Lu, 2012	Hou et al. 2012; Fei and Ye 2016
17	<i>T. maolanensis</i> Li, Wei, Cheng, Zhang & Wang, 2020	Li et al. 2020
18	<i>T. ngoclinhensis</i> Phung, Pham, Nguyen, Ninh, Nguyen, Bernardes, Le, Ziegler & Nguyen, 2023	Phung et al. 2023
19	<i>T. notialis</i> Stuart, Phimmachak, Sivongxay & Robichaud, 2010	Stuart et al. 2010
20	<i>T. obsti</i> Bernardes, Le, Nguyen, Pham, Pham, Nguyen and Ziegler. 2020	Bernardes et al. 2020
21	<i>T. panhai</i> Nishikawa, Khonsue, Pomchote & Matsui, 2013	Nishikawa et al. 2013a
22	<i>T. panwaensis</i> Grismer, Wood, Quah, Thura, Espinoza & Murdoch, 2019	Grismer et al. 2019
23	<i>T. pasmansi</i> Bernardes, Le, Nguyen, Pham, Pham, Nguyen & Ziegler, 2020	Bernardes et al. 2020
24	<i>T. phukhaensis</i> Pomchote, Khonsue, Thammachoti, Hernandez, Peerachidacho, Suwannapoom, Onishi & Nishikawa, 2020	Pomchote et al. 2020
25	<i>T. podichthys</i> Phimmachak, Aowphol & Stuart, 2015	Phimmachak et al. 2015
26	<i>T. pseudoverrucosus</i> Hou, Gu, Zhang, Zeng & Lu, 2012	Hou et al. 2012; Fei and Ye 2016
27	<i>T. pulcherrimus</i> Hou, Zhang, Li & Lu, 2012	Hou et al. 2012; Fei and Ye 2016
28	<i>T. shanjing</i> Nussbaum, Brodie & Yang, 1995	Nussbaum et al. 1995; Fei and Ye 2016
29	<i>T. shanorum</i> Nishikawa, Matsui & Rao, 2014	Nishikawa et al. 2014
30	<i>T. sini</i> Lyu, Wang, Zeng, Zhou, Qi, Wan & Wang, 2021	Lyu et al. 2021
31	<i>T. soimalai</i> Pomchote, Peerachidacho, Khonsue, Sapewisut, Hernandez, Phalaraksh, Siriput & Nishikawa, 2024	Pomchote et al. 2024
32	<i>T. sparreboomi</i> Bernardes, Le, Nguyen, Pham, Pham, Nguyen & Ziegler, 2020	Bernardes et al. 2020
33	<i>T. taliangensis</i> Liu, 1950	Liu 1950; Fei and Ye 2016
34	<i>T. tongziensis</i> Li, Liu, Shi, Wei & Wang, 2022	Li et al. 2022
35	<i>T. thaiorum</i> Poyarkov, Nguyen & Arkhipov, 2021	Poyarkov et al. 2021
36	<i>T. umphangensis</i> Pomchote, Peerachidacho, Hernandez, Sapewisut, Khonsue, Thammachoti & Nishikawa, 2021	Pomchote et al. 2021
37	<i>T. uyeno</i> i Nishikawa, Khonsue, Pomchote & Matsui, 2013	Nishikawa et al. 2013a
38	<i>T. verrucosus</i> Anderson, 1871	Anderson 1871; Fei and Ye 2016
39	<i>T. vietnamensis</i> Böhme, Schöttler, Nguyen & Köhler, 2005	Böhme et al. 2005
40	<i>T. wenxianensis</i> Fei, Ye & Yang, 1984	Fei et al. 1984; Fei and Ye 2016
41	<i>T. yangi</i> Hou, Zhang, Zhou, Li & Lu, 2012	Hou et al. 2012; Fei and Ye 2016
42	<i>T. zaimeng</i> Decemson, Lalremsanga, Elangbam, Mathipi, Shinde, Purkayastha, Arkhipov, Bragin & Poyarkov, 2023	Decemson et al. 2023
43	<i>T. ziegleri</i> Nishikawa, Matsui & Nguyen, 2013	Nishikawa et al. 2013b

closely related congeners, *T. dabienicus*, *T. gaowangjienensis*, and *T. tongziensis*, were 2.5%, 2.4%, and 1.7%, respectively, which were higher than or at a similar level to those observed among many pairs of congeners, for example, 1.2% between *T. shanjing* and *T. verrucosus* and 1.8% between *T. shanorum* and *T. ngarsuensis*

(Suppl. material 2). Similarly, the uncorrected 16S p-distances between the unnamed taxon and its most closely related congeners, *T. dabienicus* (0.9%), *T. gaowangjienensis* (0.8%), and *T. tongziensis* (0.7%), were higher than or at a similar level to those among many pairs of congeners (Suppl. material 3).

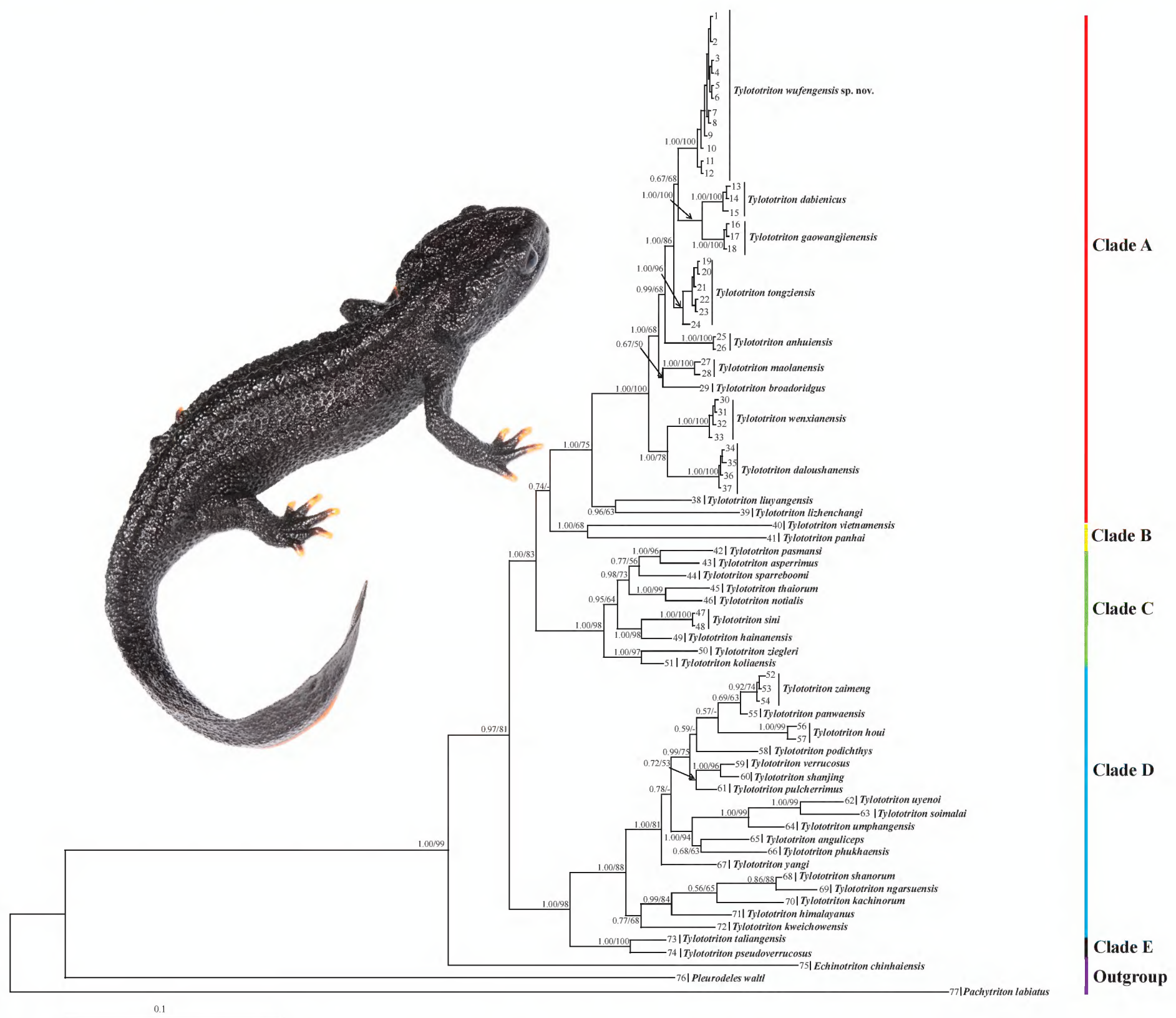


Figure 2. BI tree of the genus *Tylotriton* reconstructed based on the 16S and ND2 gene sequences. Bayesian posterior probabilities/ML bootstrap supports are shown beside each node, and “-” denotes BS < 50% or BPP < 0.60. Samples 1–77 refer to Table 1.

Divergence time estimation

The most recent common ancestor of the genus *Tylotriton* likely originated in the mid-Miocene, circa 13.98 Mya (95% highest posterior density [HPD]: 11.91–17.61 Mya). Divergence between the undescribed taxon and *T. dabienicus* and *T. gaowangjienensis* occurred at approximately 2.53 Mya (95% highest posterior density [HPD]: 1.53–3.72 Mya) (Fig. 3).

Species delimitation

BFD strongly supported the nine-species model over the seven-species model, with path-sampling (PS) marginal likelihoods favoring the former (Table 3). Similarly, bPTP analysis supported the unnamed taxon as a distinct species with a posterior probability (PP) of 0.99, rejecting the hypothesis of conspecificity with *T. dabienicus* and *T. gaowangjienensis*.

Table 3. Marginal likelihood estimation (MLE) for each species model and pairwise Bayes factor delimitation (BFD) between tested models.

Model	Species combination	MLE Path Sampling	2 ln(BF)
M1	9 species T.W+T.D+T.G+T.T+T.A+T.M+T.B+T.DA+T.WE	-5383.7	18.2
M2	7 species T.WDG+T.T+T.A+T.M+T.B+T.DA+T.WE	-5392.8	

Note: BFD interpretation is as follows: 0 < 2 ln(BF) < 2 indicates no support, 2 < 2 ln(BF) < 6 indicates positive support, 6 < 2 ln(BF) < 10 indicates strong support, and 2 ln(BF) > 10 indicates decisive support. Species abbreviations: *Tylotriton wufengensis* sp. nov. (TW), *T. dabienicus* (TD), *T. gaowangjienensis* (TG), *T. tongziensis* (TT), *T. anhuiensis* (TA), *T. maolanensis* (TM), *T. broadoridgus* (TB), *T. daloushanensis* (TDA), *T. wenxianensis* (TWE), and the combined taxon *Tylotriton wufengensis* sp. nov., *T. dabienicus*, and *T. gaowangjienensis* treated as one species (TWDG).

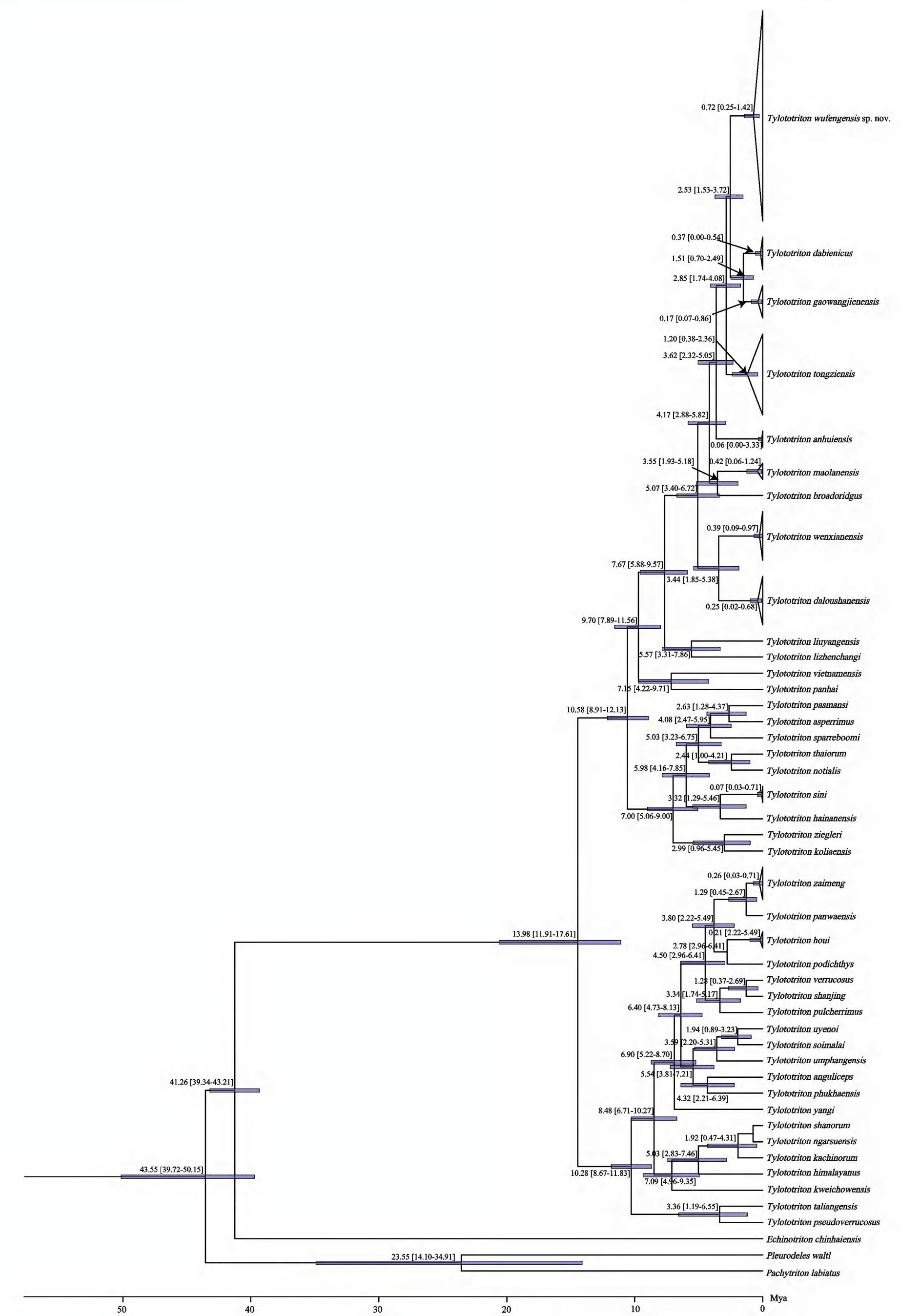


Figure 3. Time tree of the genus *Tylotriton* based on the combined mitochondrial gene sequences estimated using BEAST. The blue line at each node corresponds to the 95% highest posterior density of node ages. The bottom axis is in millions of years.

Morphological analyses

The results of ANOVA indicated that, in both males and females, the undescribed taxon differed significantly from *T. dabienicus*, *T. gaowangjienensis*, and *T. tongziensis* in multiple morphometric characters ($p < 0.05$; Suppl. material 4). In the PCA for males and females, the cumulative variance explained by the first two principal components was 71.3% and 65.2% for males and females, respectively. In two-dimensional plots of PC1 versus PC2, the undescribed taxon was separable from *T. dabienicus*, *T. gaowangjienensis*, and *T. tongziensis* in both sexes. Specifically, males of the undescribed taxon exhibited clear differentiation from *T. dabienicus* and *T. tongziensis* in the PCA (Fig. 4), whereas partial overlap with *T. gaowangjienensis* suggested limited separability in certain morphological traits. Detailed morphological comparisons further revealed distinct diagnostic characters distinguishing the undescribed taxon from its congeners.

Morphological comparisons

The undescribed taxon is phylogenetically clustered into clade A, containing *T. dabienicus*, *T. gaowangjienensis*, *T. tongziensis*, *T. anhuiensis*, *T. maolanensis*, *T. broadoridgus*, *T. daloushanensis*, *T. wenxianensis*, *T. liuyangensis*, *T. lizhengchangi*, and *T. koliaensis* (Fig. 2), and can be distinguished from these closely related species by a series of morphological characters (Suppl. material 5).

The undescribed taxon differs from *T. dabienicus* by the presence of a gular fold (vs. absent); fingers of the fore- and hindlimbs meeting when adpressed along the body (vs. not touching); vomerine teeth long and prominent, forming a “Λ” shape and separated from each other at the tips (vs. connected; Fig. 5A4, B4); and smaller TOL, HDL, TRL, ML, FIIL, and TAL in both males and females (Suppl. material 4).

The undescribed taxon differs from *T. gaowangjienensis* by fingers of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping) and by smaller HDL, TRL, and TIIL in males; it also differs by longer TOL and SVL and smaller TRL, IOD, TIIL, and IND in females (Suppl. material 4).

The undescribed taxon differs from *T. tongziensis* by fingers of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping); vomerine teeth long and prominent, forming a “Λ” shape and separated from each other at the tips (vs. connected; Fig. 5A4, C4); and by longer SVL, HDL, SL, IFE, IAE, ML, FIIL, and TL in males, as well as smaller TRL, ED, TIIL, and IND in both males and females (Suppl. material 4).

The undescribed taxon differs from *T. anhuiensis* by having the tips of the vomerine teeth disconnected (vs. connected).

The undescribed taxon differs from *T. maolanensis* by having a shorter total length (TOL 124.2–147.7 mm in males vs. 151.0–172.0 mm in males); nodule-like warts on the body sides that are continuous and indistinct (vs. separated

and distinct); and fingers of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping).

The undescribed taxon differs from *T. broadoridgus* by the tips of the fore- and hindlimbs meeting when adpressed along the body (vs. not touching); fingers of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping); and the presence of a gular fold (vs. absent).

The undescribed taxon differs from *T. daloushanensis* by the tips of the fingers extending beyond the snout when adpressed along the head (vs. between the eyes and nostrils) and by fingers of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping).

The undescribed taxon differs from *T. wenxianensis* by the presence of a gular fold (vs. absent).

The undescribed taxon differs from *T. liuyangensis* by fingertips extending beyond the snout when the forelimbs are stretched forward (vs. reaching the level of the eye) and by the tips of the fore- and hindlimbs meeting when adpressed along the body (vs. not touching).

The undescribed taxon differs from *T. lizhengchangi* by the tips of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping).

The undescribed taxon differs from *T. koliaensis* by having a longer SVL in both males and females; a gular fold present (vs. weak); the tips of the fore- and hindlimbs meeting when adpressed along the body (vs. overlapping); and nodule-like warts on the body sides that are continuous and indistinct (vs. separated and distinct).

For species within clade B, the undescribed taxon differs from *T. panhai* by having a longer SVL in females (74.9–88.1 mm vs. 71.6 mm) and by nodule-like warts on the body sides that are continuous and indistinct (vs. separated and distinct). It differs from *T. vietnamensis* by having a longer TOL in males (124.2–147.7 mm vs. 113.0–121.8 mm), nodule-like warts on the body sides that are continuous and indistinct (vs. separated and distinct), and a gular fold present (vs. absent).

For species within clade C, the undescribed taxon differs from *T. panhai*, *T. asperrimus*, *T. pasmansi*, *T. sparreboomi*, *T. thaorum*, *T. notialis*, *T. sini*, *T. hainanensis*, and *T. ziegleri* by having nodule-like warts on the body sides that are continuous and indistinct (vs. separated and distinct).

For the species within clade D, the undescribed taxon differs from *T. anguliceps*, *T. himalayanus*, *T. houi*, *T. kachinorum*, *T. kweichowensis*, *T. ngarsuensis*, *T. ngo-clinhensis*, *T. panwaensis*, *T. phukhaensis*, *T. podichthys*, *T. pulcherrimus*, *T. shanjing*, *T. shanorum*, *T. soimalai*, *T. umphangensis*, *T. uyenoii*, *T. verrucosus*, *T. yangi*, and *T. zaimeng* by having a black body except for the tips of fingers and toes, margin of vent, and ventral edge of tail (vs. having distinct yellow to reddish brown markings on the head, trunk, and tail edges in the latter).

For species within clade E, the undescribed taxon differs from *T. taliangensis* by having a shorter total length (TOL 124.2–147.7 mm in males and 126.9–155.8 mm in females vs. 186.0–220.0 mm in males and 194.0–230.0 mm in females) and by the absence of orange markings on the parotoids (vs. present). It differs from *T. pseudoverrucosus*

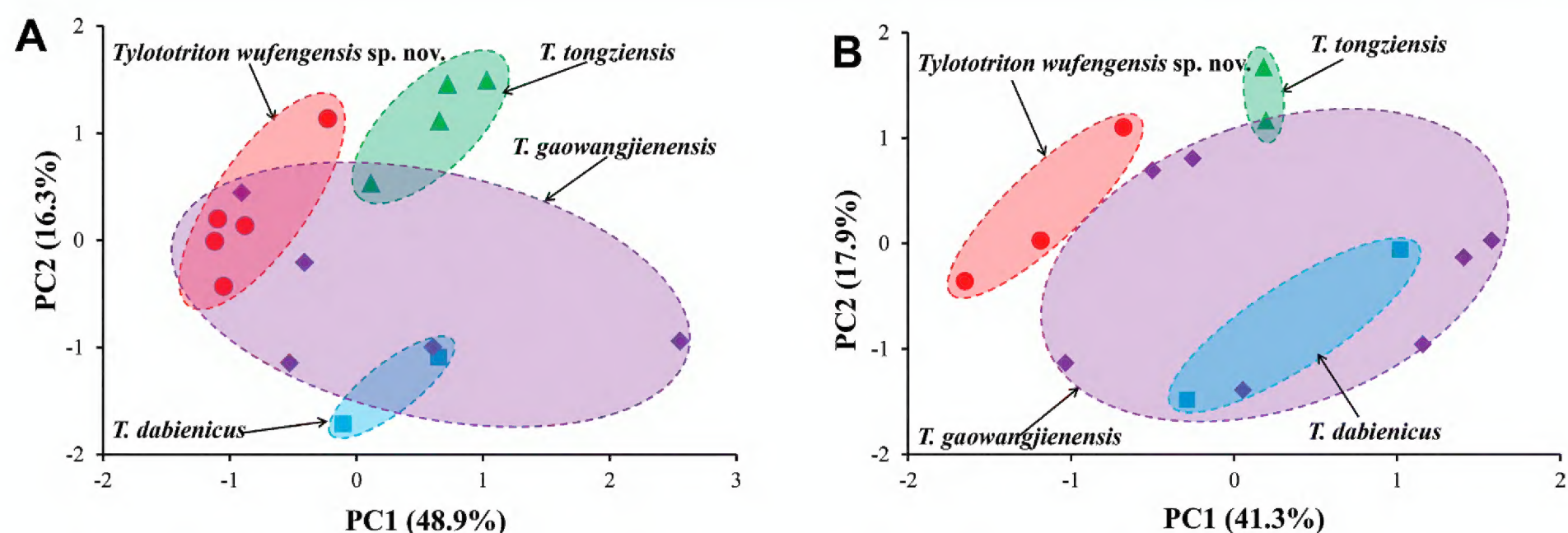


Figure 4. Plots of the first principal component (PC1) versus the second (PC 2) for *Tylototriton wufengensis* sp. nov., *T. dabienicus*, *T. gaowangjienensis*, and *T. tongziensis* in males (A) and females (B) based on principal component analysis.

by having a shorter total length (TOL 124.2–147.7 mm in males and 126.9–155.8 mm in females vs. 156.2–173.0 mm in males and 178.2 mm in females), nodule-like warts on the body sides that are continuous and indistinct (vs. separated), and a black body except for the tips of the fingers and toes, the margin of the vent, and the ventral edge of the tail (vs. distinct yellow to reddish brown markings on the head, trunk, and tail edges).

Taxonomic accounts

Based on integrative taxonomic evidence from molecular and morphological analyses, we herein describe this unidentified taxon as a new species.

Tylototriton wufengensis sp. nov.

<https://zoobank.org/43542C02-58A9-4B15-8649-56EA23413DD7>

Holotype. CIB WF20240502001 adult male, collected by Shize Li on 2 May 2024 in Wufeng County (30.187°N, 110.931°E; elevation 1045 m a.s.l.), Hubei Province, China.

Paratypes. Three females, CIB WF20240502004, CIB WF20240502007, and CIB WF20240502008, and four males, CIB WF20240502002, CIB WF20240502003, CIB WF20240502005, and CIB WF20240502006, were collected by Jing Liu on 2 May 2024. All of the paratypes were collected from the same place as the holotype.

Diagnosis. *Tylototriton wufengensis* sp. nov. is assigned to the genus *Tylototriton* based upon molecular phylogenetic analyses and the following morphological characters: presence of dorsal granules, dorsolateral bony ridges on the head, knob-like warts on dorsolateral body, and the absence of a quadrate spine.

Tylototriton wufengensis sp. nov. can be distinguished from other congeners by the following characters: (1) body size moderate (SVL 63.7–77.2 mm in males and 74.9–88.1 mm in females); (2) head width larger than head length; (3) gular fold present; (4) the tail length shorter than the

snout-vent length; (5) the distal ends and ventral surfaces of digits, peripheral area of cloaca, and the lower margin of tail orange; (6) the distal tips of the limbs just meeting when the fore and hindlimbs being pressed along the trunk; (7) fingertips reaching to the level beyond the snout when the forelimbs being stretched forward; (8) nodule-like warts on body sides that are continuous and not obvious.

Description of holotype. Adult male (Figs 5, 6), body size moderate, SVL 74.8 mm; TOL 138.5 mm; head width larger than head length (HDW/HDL = 1.13); head slightly concave on the top; snout nearly square, extending beyond the lower lip; naris nearly snout; snout truncate in dorsal view; labial fold absent; dorsolateral bony ridges on head prominent, moderately protruding, from above snout to above anterior end of parotoid, posterior ends relatively thick and scrolled inside; mid-dorsal ridge on head distinct and thin; parotoids enlarged, projecting backwards; inter-nasal space is smaller than the inter-orbital space; eyes protrude from the dorsolateral portion of the head; the oral fissure flat; the joint of jaw articulation lies posterior to the caudal margin of the eyes; fine teeth present on the edge of jaw, vomerine teeth long and prominent, form a “Λ” shape which separated from each other at the tip of the “Λ” shaped; tongue oval and nearly entirely fixed at bottom but free at both lateral edges; neck is rounded and thick, gular fold present.

Body stout; skin on lateral body with no obvious presence of small glands, glandular vertebral ridge prominent, slightly rough, and segmented, extending from top of head to base of tail, rib nodules indistinct.

Four limbs slender, fingers from fore- and hindlimbs meeting when addressed along body; tips of fingers beyond snout when addressed along head; relative fingers length III > II > I > IV, and relative toes length III > IV > II > I > V; both fingers and digits are with none webbed.

The tail length shorter than the snout-vent length; the tail laterally compressed, thin, and tip acuminate, distinctly thin and high; the tail height is greater than the width at the tail base, and the tip of the tail resembles a sword shape; the cloaca long and narrow, and the cloacal region slightly bulbous.

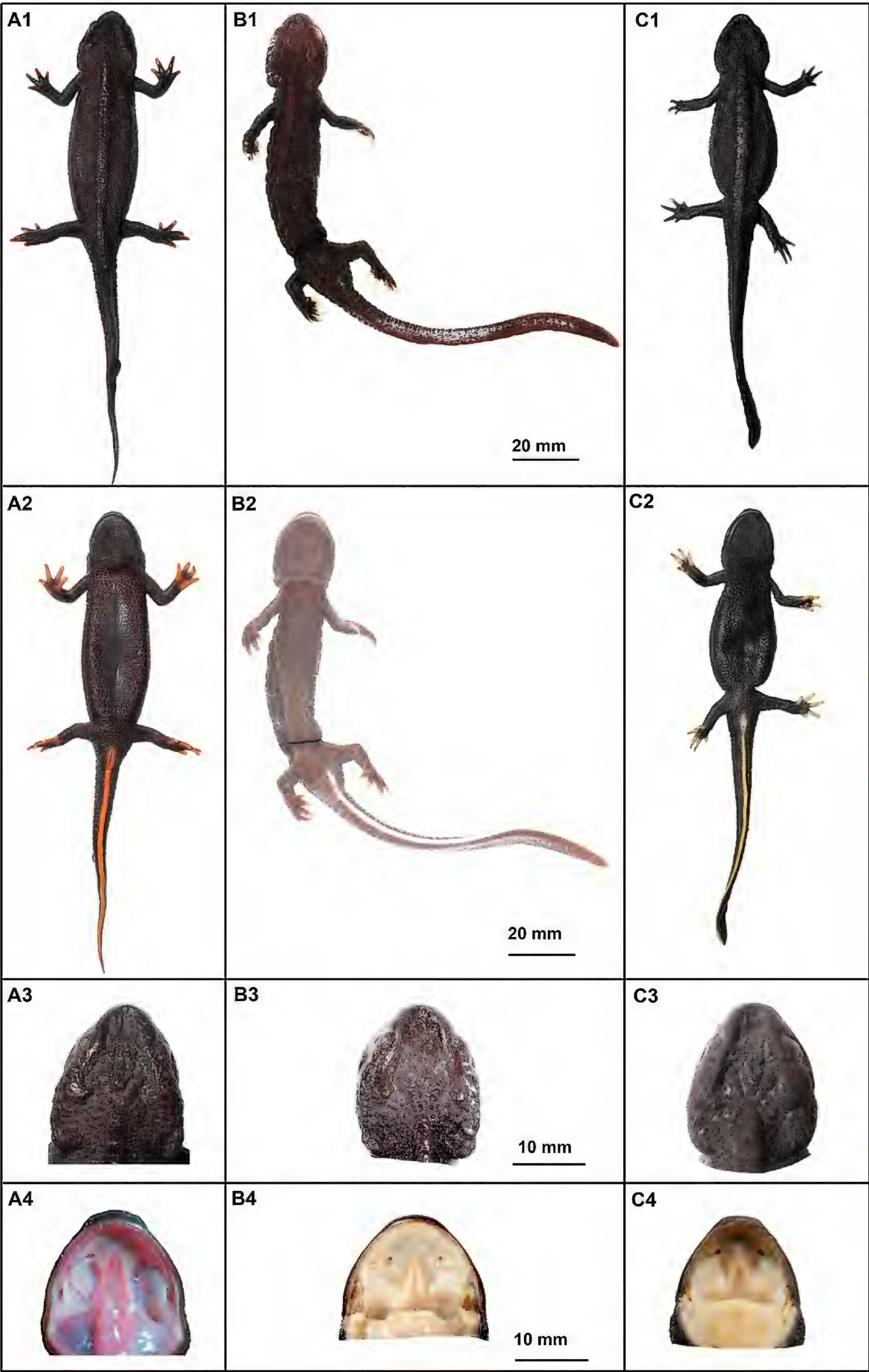


Figure 5. Photographs of the holotype specimen of *Tylototriton wufengensis* sp. nov. and specimens of its closely related species. **A.** Holotype specimen CIB WF20240502001 of *Tylototriton wufengensis* sp. nov.; **B.** Topotype specimen CIB 8042905 of *T. dabienicus*; **C.** Topotype specimen CIB WB2020190 of *T. tongziensis*. **A1, B1, C1.** Dorsal view of the whole body; **A2, B2, C2.** Ventral view of the whole body; **A3, B3, C3.** Dorsal view of the head; **A4, B4, C4.** View of the oral cavity. (Photos A1–A4, B1–B4, C1–C4, and D1–D4 by Shengchao Shi.)



Figure 6. Photographs of the holotype CIB WF20240502001 of *Tylototriton wufengensis* sp. nov. in life. **A.** Lateral view; **B.** Dorsal view; **C.** Ventral view; **D.** Tail; **E.** Lateral view of the head; **F.** Dorsal view of the head; **G.** Ventral view of the hand; **H.** Ventral view of the foot; **I.** Cloaca. (Photos **A–I** by Shengchao Shi.)

The skin rough, body covered with small tubercles; the labial margin, distal limbs, ventral limbs, and ventral edge of the tail smooth; on the lateral dorsum of the body, tubercles small, appearing to form lines that extend from the shoulder to the base of the tail, and the nodule-like tubercles continuously distributed with no clear separation between neighboring tubercles; the tubercles on pharyngeal small, distinct and sparse, and tubercles on the abdomen relative large and dense.

Coloration in life. In life, the overall body color is black. Orange coloration is present on the dorsal and ventral surfaces of the fingers and toes, the peripheral area of the cloaca, and the ventral edge of the tail (Fig. 6).

Color in preservative. The specimen in preservative is blackish-brown. The orange coloration of the distal ends and ventral surface of digits, the peripheral area of the cloaca, and the ventral edge of the tail fades to light orange (Fig. 5).

Variation. Measurements of all specimens are listed in Table 3 and Suppl. material 1. All specimens were very similar in morphology. But in CIB WF20240502004, there is an orange-red protrusion behind the parotoid gland (Fig. 7B); cloaca and the ventral edge of tail disconnected (Fig. 7D).

Secondary sexual characters. In male, the cloacal orifice long slit and has papilla on inner cloacal wall (Fig. 6I) and in female, cloacal hole is short and its inner cloacal wall has no papilla (Fig. 7D).

Distribution and habitats. At present, *Tylototriton wufengensis* sp. nov. is known only from Wufeng County, Hubei Province, China. The population inhabits mountain ponds at an altitude of approximately 1000 m, where accumulated rocks are present. During the day, *Tylototriton wufengensis* sp. nov. hides under these rocks and becomes active at night. Near these ponds inhabited by *Tylototriton wufengensis* sp. nov., there are farmlands (Fig. 8). *Polypedates megacephalus* Hallowell, 1861, *Pelophylax nigromaculatus* (Hallowell, 1860), and *Zhangixalus chenfui* (Liu, 1945) were also found at the type locality of the new species.

Etymology. The specific epithet “wufeng” refers to the distribution of this species, Wufeng County, Hubei Province, China. We propose the common name “Wufeng Knobby Newt” and its Chinese name as Wu Feng You Yuan (五峰疣螈).

Discussion

Using mitochondrial and nuclear gene data, Wang et al. (2018) identified the *Tylototriton* population from Wufeng County, Hubei Province, as a distinct cryptic lineage. Through integrative taxonomic approaches, including morphological comparisons, phylogenetic analyses, divergence time estimation, and species delimitation methods, we validate its classification as a new species. Although mitochondrial 16S genetic divergence is low among closely related congeners (0.7–0.9%), the integration of multilocus evidence (e.g., ND2 divergence of 1.7–2.5%) and divergence time analyses is consistent with speciation driven by late Miocene geographic isolation (Wang et al. 2018). This underscores the necessity of combining multiple lines of evidence to resolve species boundaries in morphologically conservative taxa (Cheng et al. 2025). We formally describe this lineage as *Tylototriton wufengensis* sp. nov., addressing the cryptic diversity highlighted by Wang et al. (2018).

Despite significant progress in delimiting *Tylototriton* diversity, cryptic species complexes likely remain undescribed. Wang et al. (2018) identified several cryptic lineages, such as *T. wenxianensis* lineage 3 and *T. dabienicus* lineage 2, which have since been formally described (Qian et al. 2017; Li et al. 2020). However, recent studies continue to reveal species outside the scope of earlier frameworks, such as *T. tongziensis* from



Figure 7. Color variation in *Tylototriton wufengensis* sp. nov. of the female specimen CIB WF20240502004. A. Dorsal view; B. Dorsal view of the head; C. Ventral view; D. Cloaca. (Photos A–D by Shengchao Shi.)

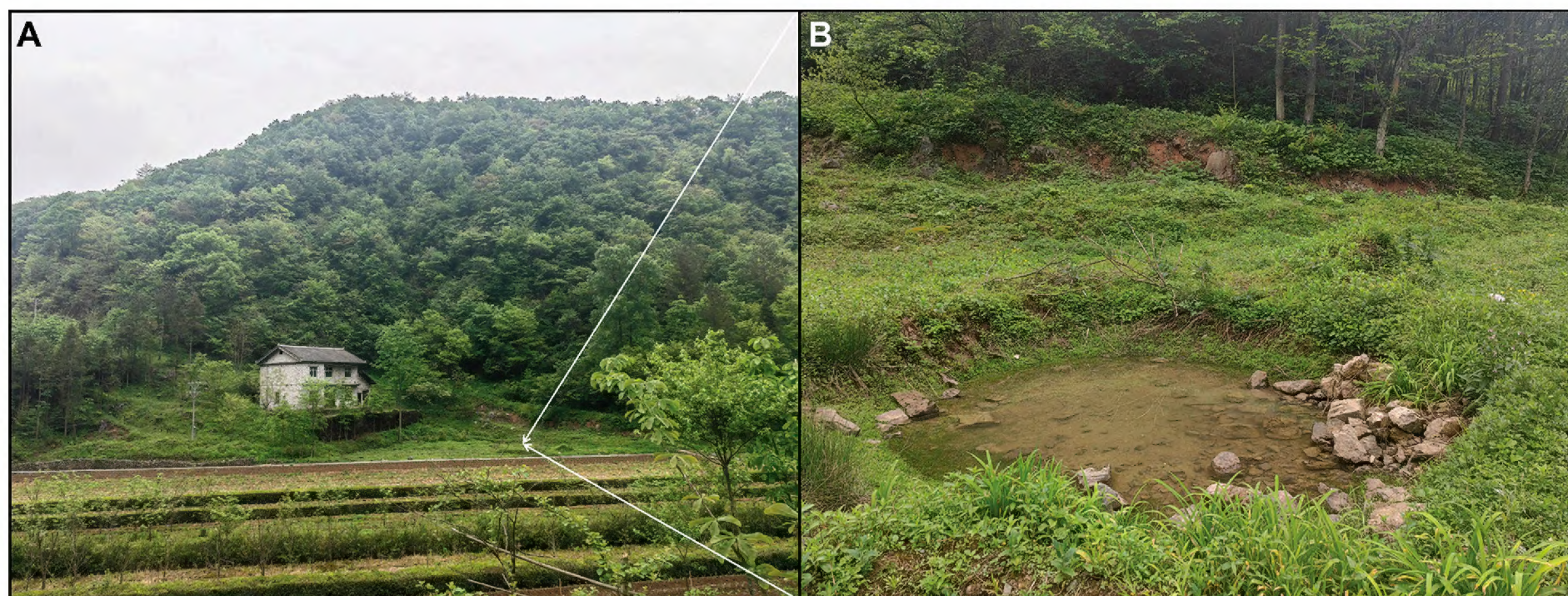


Figure 8. Habitat of *Tylotriton wufengensis* sp. nov. at the type locality, Wufeng County, Hubei Province, China. **A.** Landscape; **B.** Mountain ponds occupied by the species. (Photos **A** and **B** by Shize Li.)

Guizhou (Li et al. 2022), indicating that the diversity of the genus remains underestimated. These discoveries are concentrated in under-surveyed regions with complex topography, such as the karst landscapes of southern China, where geographic barriers and microhabitat specialization promote speciation (Wen et al. 2022; Jin et al. 2022; Zhao et al. 2022; Yang et al. 2024). Further integrative studies, particularly in biogeographic transition zones such as the Wuling–Daba Mountain corridor, are critical for uncovering remaining cryptic lineages.

Tylotriton wufengensis sp. nov. faces significant anthropogenic threats due to its restricted range and lack of protection. The type locality is surrounded by villages and agricultural lands, exposing the species to habitat fragmentation, water pollution from pesticides, and road construction. Unlike congeners inhabiting protected reserves (e.g., *T. maolanensis* in the Maolan Nature Reserve; Li et al. 2020), this species lacks formal conservation safeguards. Based on IUCN Red List criteria (B1ab(iii)+2ab(iii); restricted distribution, declining habitat quality, and absence of protected areas), we recommend classifying this species as Vulnerable (VU) (IUCN 2012). Immediate conservation actions should include establishing a micro-reserve at the type locality to mitigate habitat degradation, engaging local communities in sustainable land-use practices, and initiating population monitoring to assess demographic trends. This discovery also highlights broader gaps in amphibian documentation in Hubei Province, where only one new species has been described in the past 5 years (Shen et al. 2020). Targeted surveys in the Wuling–Daba Mountain biodiversity hotspot are urgently needed to address these gaps.

Acknowledgments

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Supplementary material 1

Individual measurements of adult specimens of *Tylototriton wufengensis* sp. nov., *T. dabienicus*, *T. gaowangjienensis*, and *T. tongziensis*

Authors: Shize Li, Shengchao Shi, Jing Liu, Zhonghao Luo, Jiajun Wang, Linhong Liao, Yeqing Wang, Renhu Gong, Jun Wu, Bin Wang

Data type: xlsx

Explanation note: Units are given in mm. Abbreviations for morphological characters are provided in the Materials and methods section.

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Link: <https://doi.org/10.3897/zse.102.173283.suppl1>

Supplementary material 2

Uncorrected *p*-distances between species of *Tylototriton* based on ND2 gene sequences

Authors: Shize Li, Shengchao Shi, Jing Liu, Zhonghao Luo, Jiajun Wang, Linhong Liao, Yeqing Wang, Renhu Gong, Jun Wu, Bin Wang

Data type: xls

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Supplementary material 3

Uncorrected *p*-distances between species of *Tylototriton* based on 16S gene sequences

Authors: Shize Li, Shengchao Shi, Jing Liu, Zhonghao Luo, Jiajun Wang, Linhong Liao, Yeqing Wang, Renhu Gong, Jun Wu, Bin Wang

Data type: xls

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Supplementary material 4

Measurements of adult specimens

Authors: Shize Li, Shengchao Shi, Jing Liu, Zhonghao Luo, Jiajun Wang, Linhong Liao, Yeqing Wang, Renhu Gong, Jun Wu, Bin Wang

Data type: xlsx

Explanation note: Measurements of adult specimens of *Tylototriton wufengensis* sp. nov. and results of one-way ANOVA, with *p*-values for morphometric comparisons among *Tylototriton wufengensis* sp. nov. (TW), *T. dabienicus* (TD), *T. gaowangjienensis* (TG), and *T. tongziensis* (TT). Units are given in mm. Abbreviations for morphological characters are provided in the Materials and methods section.

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Supplementary material 5

Morphological characters used for comparisons among *Tylototriton* species

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Data type: xlsx

Explanation note: “/” indicates information not known or not clearly defined in the literature.

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